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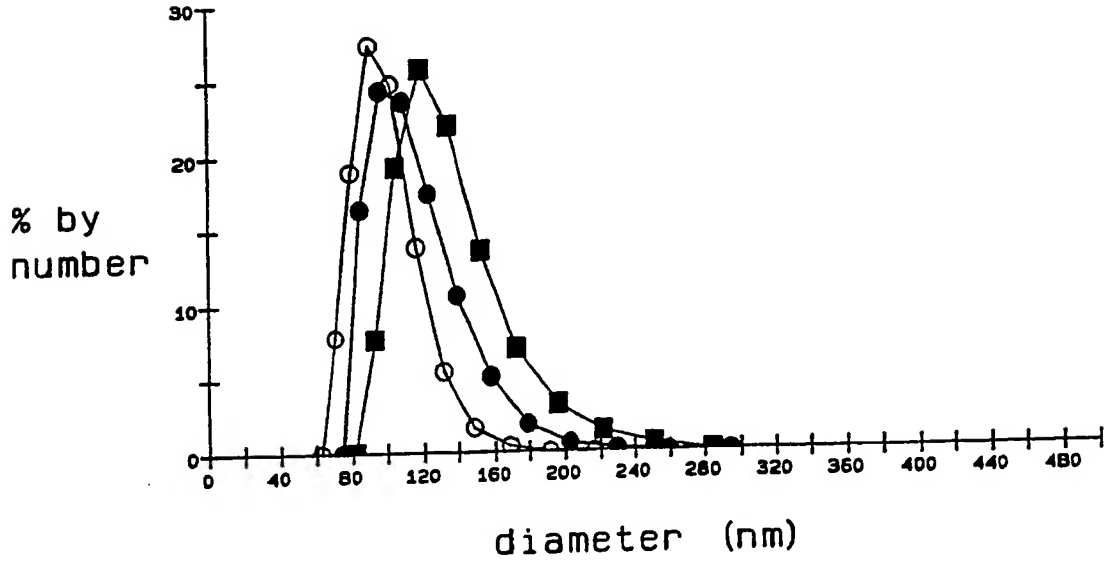
(54) **New compositions**

(57) The present invention provides micelles which avoid uptake by the mononuclear phagocyte system formed from a block copolymer having one hydrophobic block and one or two hydrophilic blocks where a drug or label is chemically attached to the core-forming hydrophobic block.

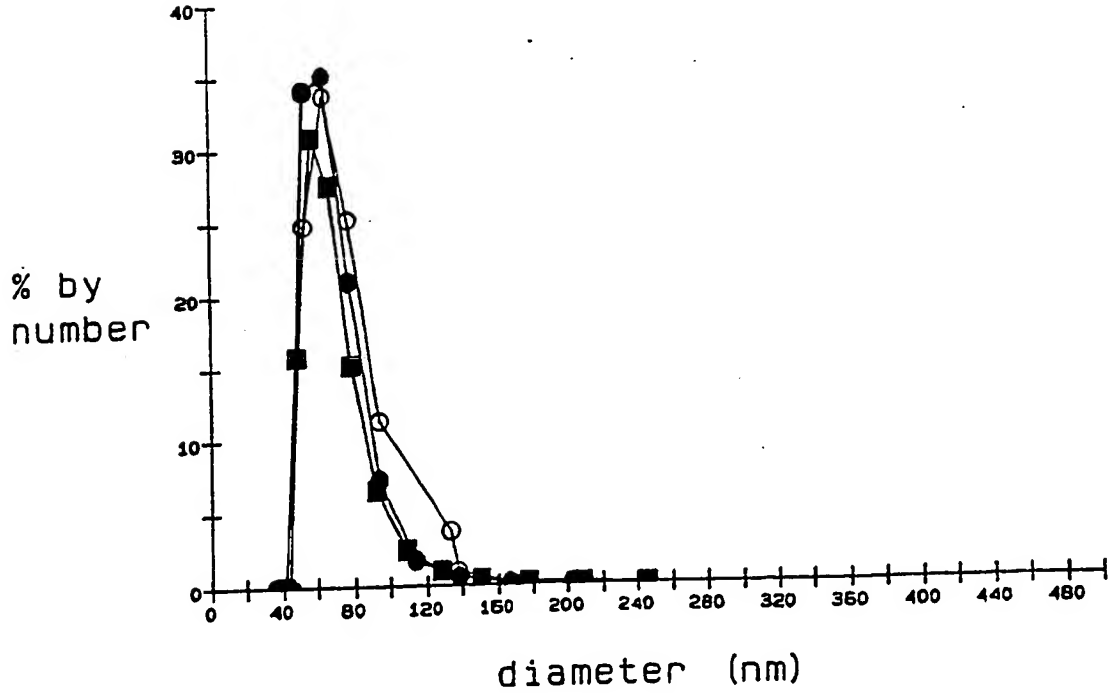
GB 2 240 547 A

Fig 1

THE EFFECT OF THE ADDITION OF FIBRINOGEN
ON THE SIZE OF POLYSTYRENE PARTICLES.



THE EFFECT OF THE ADDITION OF FIBRINOGEN
ON COPOLYMER 9 AGGREGATES.



New Compositions

The present invention relates to biocompatible polymeric delivery systems for drugs.

Conventional, small molecular weight drugs are distributed throughout the body by physico-chemical processes such as convective fluxes, diffusion and partition. It is a fact that large molecules (i.e. larger than about 500 Dalton) cross biological membranes by the process known as endocytosis. Endocytosis can in principle be used for delivering drugs to a selected population of cells by attaching drugs to macromolecules to alter the drug biodistribution.

It has been proposed to use colloidal particles in the form of microspheres, microcapsules, emulsions and liposomes for directing drugs contained therein to specific sites in the body, a concept generally known as drug targetting.

Generally, colloidal materials that are placed into the vascular compartment of the body are removed from circulation by the monocytic phagocyte system (MPS). The rate of removal of colloidal particles has been shown to be related to factors such as size, chemical composition of the particle surface, the thickness of the hydrated surface layer relative to the particle core size, surface charge, and the dose. It can also be different in various animal species, and can vary according to the physiological state of the species.

For particles that are not physically entrapped, it is known that various manipulations of particle surfaces lead to qualitative and quantitative alterations in the adsorption of proteins to particles (so called opsonization) and consequently in the macrophage uptake. Particles coated with either neutral, negative or positive macromolecules can be taken up by different organs at different rates. Synthetic and natural materials that have been used for such surface modifications include poly(oxyethylene), poly(N-[2-hydroxypropyl]methacrylamide), poly(vinylpyrrolidone), Poloxamers and Poloxamines (block copolymers of poly(oxyethylene) and poly(oxopropylene), albumin, immunoglubulin G and carboxy-methylcellulose.

Modification of surfaces of colloidal particles by either adsorption or chemical grafting of polymers alters the biological fate of the colloids, but these approaches have a number of limitations. For strong adsorption of polymers, the particle core needs to be selected to provide sufficient "anchoring" for the chains. All adsorption processes are in principle reversible and this can lead to changes in the composition of the adsorbed layer, due to

competitive desorption, in the complex biological environment. Due to steric constraints placed on the structure of well-adsorbing block copolymer, it may be difficult to obtain an adsorbed layer of sufficient chain density and thickness.

Chemical grafting of polymer chains to surface requires that suitable reactive groups are available at the particle surface to react with the reactive groups of the incoming polymers. Both due to the distribution of the reactive groups at the surface, and due to the steric hindrance caused by the initial number of chains attached to the surface, the formation of a complete surface layer of grafted polymer chains is difficult or even impossible to achieve.

Additionally, yet unsolved challenges of these approaches relate to the eventual incorporation of drugs into the particles and their ultimate release.

We believe that the use of vehicles that avoid the MPS is crucial for the development of site specific drug delivery systems.

We have now developed sterically-stabilised colloidal particles from block copolymers which are biocompatible in so far as they do not interact with plasma proteins, adhere minimally to macrophages and consequently are not taken up (phagocytosed) by the cells of the MPS (e.g. kupffer cells). The sum of these properties results in these particles being able to avoid the sequestering action of e.g. the liver and spleen, and therefore can reach other parts of the body for the purpose of drug delivery.

Accordingly the present invention provides micelles which avoid uptake by the MPS formed from a block copolymer having one hydrophobic block and one or two hydrophillic blocks where a drug or label is chemically attached to the core-forming hydrophobic block.

The invention also provides a method of making micelles having a hydrophobic core and a hydrophilic outer layer comprising making a block copolymer having a hydrophobic block and one or two hydrophilic blocks where a drug or label is chemically attached to the hydrophobic block and then adding the copolymer to water and allowing the micelles to form.

The drug or label may be attached to a block which is already hydrophobic or it may be attached to a block which is initially hydrophilic but which becomes hydrophobic when the drug is attached.

The micelles of the present invention are discrete, uniform and stable.

The block copolymers can be used to form monolithic colloidal particles by chemically cross-linking the core of the micelles. During chemical cross-linking other materials including a drug or label may be incorporated into the particles and can be retained within the particles under physiological conditions.

The resulting micelles and particles are colloidally stable under the conditions prevailing in the vascular compartment of the body. Further they do not interact with proteins and circulating cells in such a way that would lead to their removal from circulation. They also have a long circulating half-life after intravenous administration to experimental animals (mice and rats).

The hydrophilic block (or blocks) may be a polyamino acid such as poly(proline), poly(hydroxyproline) or poly(oxazoline) or a polyoxyethylene block which may have a weight average molecular weight of at least 500 and preferably at least 1000.

The hydrophobic block may be composed of a hydrophobic block having reacting sites to which a drug or label may be attached or it may be a hydrophilic block having reacting sites to which a drug or label may be attached and thereby convert the block into a hydrophobic one. The hydrophobic block may be derived from e.g. polyisoprene, poly(amino acids), or poly(saccharides) such as polyglutamic acid, polylysine and dextran. The weight average molecular weight of the hydrophobic block may be at least 2000, preferably at least 3000.

The copolymers may be produced by making the hydrophobic block by polymerisation of the selected monomer. The drug or label is then reacted with the polymer followed by reacting one or both ends of the polymer with e.g. ethylene oxide to build one or two hydrophilic blocks. Alternatively a drug or label may be attached to a pre-formed block copolymer as described above. The resulting block copolymer is then added to water to form the micelles.

The amount of polymer added to water should be sufficient to exceed the critical micelles concentration (CMC) for the polymer concerned. For the polymers we have tested based on polyoxyethylene and polyisoprene blocks we have found the CMC to be extremely low, typically less than 1×10^{-11}M.

The invention is illustrated by the following Examples which, as is common in the area of drug targetting, use model compounds to show the fate of the micelles.

Examples 1 to 10: A series of block copolymers of the general structure

POE-b-PI-b-POE

are prepared, where POE is polyoxyethylene and PI is polyisoprene. These are prepared from isoprene and ethylene oxide in tetrahydrofuran as solvent and using potassium naphthalide as initiator. The reaction is carried out at -50°C for 3 hours and then at ambient temperature for about 60 hours.

The copolymers are isolated and then dissolved in tetrahydrofuran to form 0.1 weight % solutions. The solutions are added to water and the tetrahydrofuran removed by rotary evaporation. The size of the resulting micelles and their polydispersity was determined by Photon Correlation Spectroscopy (PCS). The results and the weight average molecular weight data (M_w) for the copolymers are given in Table 1.

Table 1

Example	Mw PI block	Mw Copolymer	Diameter (nm)	Polydispersity
1	40000	62300	52.4	0.44
2	6100	12500	114.3	0.44
3	114700	81000	221.8	0.44
4	18500	38000	117.2	0.22
5	8500	21000	52.7	0.17
6	3000	5700	19.1	0.40
7	4050	10100	47.3	0.22
8	12000	22500	95.5	0.30
9	26800	34000	173.2	0.39
10	16000	33600	155.7	0.42

Example 11: To 70 ml of a 0.1 % by weight solution of the copolymer of Example 1 in water is added 1 mg of 2,2'-azobisisobutyronitrile (AIBN) and mixed for 4 hours. The solution is exposed to UV light from a high pressure mercury lamp for 2 hours, in order to cross-link the copolymer. The diameter of the particles and their polydispersity is:-

	Diameter (nm)	Polydispersity
Copolymer	110.7	0.149
Copolymer + AIBN	110.3	0.143
After cross-linking	95.3	0.142

Example 12: Example 11 is repeated using a 0.5 % by weight solution of the copolymer of Example 6, with the following results.

	Diameter (nm)	Polydispersity
Copolymer	45	0.45
Copolymer + AIBN	46	0.36
After cross-linking	28	0.30

Example 13: To 70 ml of a 0.1 wt % solution of the Copolymer of Example 1 in water is added 70 μl of styrene containing 1 wt % of AIBN and mixed for 4 hours. The solution is exposed to UV light (nm) for 2 hours.

IR spectroscopy shows that the styrene is incorporated into the particles. The particles have a diameter of 115 nm and a polydispersity of 0.133. The particles are stable to sonication and contain polystyrene after hexane extraction.

Near-monodisperse colloidal particles are produced.

Example 14: The ability of the particles of Example 1 not to bind proteins is demonstrated by measuring, by light scattering, the extent of aggregation (as reflected by an average size change) on incubating particles with fibrinogen, a protein known to be involved in

opsonizing foreign particles in the body.

The results are shown in Fig 1 which also gives the results of a similar experiment with polystyrene particles.

Example 15: Example 13 is repeated using C^{14} labelled styrene. The uptake of the particles by isolated human monocytes is determined. It is found that only about 2 % of the available "dose" is associated with the actively phagocytosing cells after 2 hours.

A similar experiment carried out using poly(methacrylate) particles results in over 80 % association in 2 hours.

Example 16: A sample of the C^{14} labelled particles used in Example 15 are administered intravenously to mice and rats and a sample of a similar C^{14} labelled compound based on the copolymer of Example 2.

The blood levels in mice after intravenous administration expressed as % dose are:-

	2 hr	24 hr	96 hr	168 hr
Example 1	31.4	37.7	7.5	0.4
Example 2	93.9	88.5	7.7	0.9

Claims

1. Micelles which avoid uptake by the mononuclear phagocyte system formed from a block copolymer having one hydrophobic block and one or two hydrophilic blocks where a drug or label is chemically attached to the core-forming hydrophobic block.

2. Micelles as claimed in claim 1 in which the drug or label is attached to a block which is already hyrophobic or it is attached to a block which is initially hydrophilic but which becomes hydrophobic when the drug or label is attached.

3. Micelles as claimed in claim 1 or 2 in which the hydrophilic block(s) is a polyamino acid block or a polyoxyethylene block having a weight average molecular weight of at least 500.

4. Micelles as claimed in claim 3 in which the polyoxyethylene block has a weight average molecular weight of at least 1000.

5. Micelles as claimed in any preceding claim in which the hydrophobic block is derived from polyisoprene, poly(amino acids), or poly(saccharides).

6. Micelles as claimed in any preceding claim in which the hydrophobic block has a weight average molecular weight of at least 2000.

7. Micelles as claimed in claim 6 in which the hydrophobic block has a weight average molecular weight of at least 3000.

8. Micelles as claimed in claim 1 substantially as hereinbefore described with reference to any one of the foregoing Examples.

9. The use of micelles as claimed in any preceding claim as carriers for drugs on labels.

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